Idaho Disease

BULLETIN

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West Nile Virus in Idaho

Locally-acquired West Nile virus (WNV) infections began appearing in Idaho during the fall of 2004, ultimately affecting 11 counties with three symptomatic people, seven positive birds and 22 positive horses. Surveillance occurred statewide; however, the virus was only found in southern and southwestern regions of the state. It has been shown, by observing the behavior of WNV in other affected states, that a surge in case-counts during the second year after evidence of the virus is discovered should be expected; therefore, 2005 should see a rise in cases for Idaho.

WNV infections are reportable in Idaho. To learn more about the varying clinical manifestations of WNV go to www.cdc.gov or www.westnile.idaho.gov.

The "Fight the Bite" community education campaign will again be used this season. Free copies of brochures in English and Spanish and posters on WNV risk factors may be acquired by contacting your district health department.

Laboratory testing for WNV

Acute and convalescent serum samples taken 7 to 14 days apart are ideal for WNV diagnosis. IgM levels in serum or CSF may not be detectable until after the first week of clinical illness. Diagnosis of WNV may include one of the following: the detection of a 4-fold or greater change in WNV-specific serum neutralizing antibodies in paired sera; isolation of WNV from or detection of viral antigens or genomic sequences in tissue, blood, CSF; WNV-specific IgM in CSF; or WNV-specific IgM antibodies confirmed by the presence of WNV-specific IgG antibodies in the same or later serum sample by another assay. Due to the potential longevity of WNV-specific IgM, paired sera would help solidify the diagnosis. The Idaho Bureau of Laboratories (IBL) will test serum specimens for IgG or IgM WNV-specific antibodies. IgM-positive samples will also be evaluated for cross-reactivity to the St. Louis encephalitis virus (SLE). CSF is generally tested for WNV-specific IgM antibodies only. If more than one ml of CSF is received, then the sample also can be evaluated for SLE-specific IgM antibodies.

A number of commercial laboratories do offer WNV serologic testing. Healthcare providers are encouraged to utilize commercially available testing for suspected cases of West Nile fever. The IBL serologic testing is offered preferentially for those with neurologic manifestations of WNV.

Serologic Testing for Infectious Diseases

Introduction

Serologic tests are frequently requested to aid in the diagnosis of infectious diseases; however, occasionally inappropriate tests are requested, adequate samples are not obtained, or test results are misinterpreted or confusing. The goal of this article is to improve the utility of serologic testing for

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infectious diseases in Idaho.

Antibody basics — a refresher

Serologic tests for diagnosing infectious diseases evaluate IgG, IgM, or, rarely, IgA. IgG comprises about 80–85 percent of the total immunoglobulins in the body and has a half-life of 21–23 days. Most bacterial, virus-neutralizing, or precipitating antibodies, hemagluttinins, and hemolysins are IgG. IgG is the only immunoglobulin to cross the placenta: at birth, most IgG in the newborn's serum is from the mother. IgM comprises about ten percent of total immunoglobulin and has a half-life of 5–6 days. IgM is the antibody most often formed in response to stimulus by gram-negative bacteria, and is most often the first antibody to appear after a primary antigenic stimulus. There are two types of IgA: serum and secretory. Serum IgA comprises about six percent of total immunoglobulin and has a half-life of 5–6.5 days. Its function is not well understood. In secretions (e.g., CSF, synovial fluid, colostrum, respiratory mucus, *etc*.), IgA serves as the first line of defense against invasion of microorganisms; its concentration in secretions is much higher than that of IgG or IgM.

Free antibody is usually detectable in the blood from 5–10 days after a primary antigenic stimulus. The total antibody titer gradually increases over a few days to a few weeks, plateaus, then begins to drop. IgM appears first, then IgG. In a secondary response (AKA memory, anamnestic, or booster response), there is at first a sharp drop in circulating antibody because it is complexing with antigen. Usually within 2–3 days the titer increases and continues to rise for several days, ultimately exceeding the titer attained in the primary response. The secondary response produces more IgG than IgM. Secondary responses may be induced by cross-reactive antigens and may be repeated many times, even years after a primary titer has dropped to zero.

Sampling and sample submission

In general, serum for detection of antibodies should be drawn as early as possible during the acute phase of illness or when first discovered and again during the convalescent period, usually two weeks later. When the first specimen is submitted, be sure to notify the laboratory that you expect to submit a convalescent phase sample. Single samples may be sufficient for diagnosis of some diseases. (e.g., IgM for hantavirus, hepatitis A, measles, mumps, or rubella; elevated serum antibody titers for *Yersinia pestis* or *Franciscella tularensis* in a patient with no history of vaccination). Typically, samples are sent to commercial laboratories for testing. The IBL is mandated to support state and local health departments in their duties and supply testing which supports and confirms private physicians and clinical laboratory efforts. The IBL supports testing during disease outbreaks, provides tests that are not commercially available, (e.g, SARS serology and PCR, *Norovirus* PCR, West Nile virus PRNT, avian influenza PCR, orthopox PCR) and forwards samples to the CDC for specialized testing (e.g., confirmation of rickettsial titers, enterovirus subtyping, *Salmonella* phage typing, rabies virus subtyping, influenza virus subtyping, multiplex PCR on genital ulcer swabs). The IBL sampling and submission guide can be found by navigating through the IDHW website at www.healthandwelfare.idaho.gov.

General test interpretation

For most pathogens, a fourfold increase in the patient's titer, for example, from a positive result of 1:8 to a positive result or 1:32 over two to four weeks is considered to be diagnostic of a current infection. For diagnosis of Q-fever, samples are best taken 3–6 weeks apart.

Testing of serum samples for comparative purposes (e.g., acute and convalescent samples) should be done with the same technique, at the same laboratory, preferably on the same day with the same equipment, to avoid intra- and inter-laboratory variability that

could result in a false difference between titers and subsequent errors in diagnostic interpretation.

Clinical diagnosis vs. reportable diseases

The Council of State and Territorial Epidemiologist and the CDC collaborate to establish case definitions for infectious diseases under public health surveillance (available at the following website http://www.cdc.gov/epo/dphsi/casedef/index.htm). This website provides updated uniform criteria for public health professionals to use when reporting nationally notifiable infectious diseases. It should be recognized that physicians may diagnose and treat a case of infectious disease without the case meeting these criteria; cases that meet diagnostic criteria but do not meet public health case definitions may be reported as "suspected" cases to CDC.

Diagnostic testing issues related to specific diseases

1. Syphilis

An ongoing syphilis outbreak in southwestern Idaho has drawn attention to the importance of correctly ordering serologic tests for syphilis. Syphilis testing is confusing because of the variety of tests available and the different purposes to which they are used. Common non-treponemal syphilis screening tests are USR, RPR, and VDRL. Common treponemal confirmatory tests include FTA-abs, TP-PA, and MHA-TP. Blood banks usually screen using the automated PK-TP, then follow up with the RPR and Captia Syphilis G EIA for confirmation. For persons presenting with symptoms suggestive of syphilis, a nontreponemal screening test should be ordered, and confirmed with a treponemal test if positive. Testing for evaluation of mother-infant transmission and treatment efficacy is the most problematic in Idaho. Idaho law mandates screening pregnant women for syphilis at their first prenatal visit or within 15 days thereafter. For women at high risk for acquiring syphilis during pregnancy, testing in the 3rd trimester and at delivery is recommended. In mothers with suspected syphilis or positive serologic tests during pregnancy, specimens should be collected from the mother and infant at delivery for comparison. A four-fold or higher neonatal titer compared with the maternal titer suggests neonatal infection, although an infected neonate may have a lower titer than the mother or no titer at all. Nontreponemal antibody titers in neonates should decline by 3 months of age and should be nonreactive by 6 months of age if the infant's reactive test result was caused by passive transfer of maternal IgG antibody. Testing is recommended for seroreactive infants every 2–3 months until the test becomes nonreactive or the titer has decreased fourfold. Because titers obtained from different types of tests (e.g., VDRL, RPR, USR), are *not* comparable (for example, titers are generally two- to four-fold higher on the RPR card test than in the VDRL), a true difference in titer may not be detected, or a false difference in titer may be observed if the same type of test is not ordered for both mother and infant, or for each sequential test on the infant. The same problem arises with sequential testing for follow-up on adequacy of treatment for all patients. The same type of test must be requested from the same laboratory for proper comparison of titers. If you are unsure which test was performed previously, please verify the test type and laboratory prior to submitting samples for comparison. Note that infection with HIV may alter the clinical presentation and performance of serologic tests for syphilis. The US Public Health Service Task Force's *Guide to Clinical Preventive Services, Second Edition* has an excellent discussion on syphilis screening at http://cpmcnet.columbia.edu/texts/gcps/gcps0036.html.

2. Pertussis

CDC requires a positive PCR or culture for confirmation of a pertussis case for public health surveillance purposes. Serologic tests that are not FDA approved are commercially available to clinicians; however, caution should be used in making the diagnosis of pertussis infection with serology alone because antibodies to filamentous

hemagglutinin and pertactin due to infection with *B. parapertussis* or other *Bordetella* species can also occur. In addition, detectable levels of IgG antibodies to *B. pertussis* may be seen in the serum of vaccinated individuals of all ages and in early infancy as the result of placental transfer. IgG antibodies can only be used for diagnosis of active infection when paired sera are available and a rise in antibody level can be demonstrated. A significant rise may not always be demonstrated as peak levels of IgG may be reached before the first sample is collected; therefore, IgA and IgM antibody levels should be measured to help diagnose active disease by serology. Please contact your diagnostic laboratory for help with interpretation of test results.

3. Varicella

For the diagnosis of acute varicella infection, serologic confirmation would include a significant rise in varicella IgG by any standard serologic assay. The CDC states that testing using commercial kits of IgM antibody is not recommended because available methods lack sensitivity and specificity, and that false positive IgM results are common in the presence of high IgG levels. The National VZV (varicella zoster virus) Laboratory at CDC has developed a reliable IgM capture assay; e-mail vzvlab@cdc.gov for details about collecting and submitting specimens for testing.

Routine postvaccination serologic testing is not recommended for varicella because of the potential for false negative results. Some evidence suggests that the latex agglutination method may result in false positive tests that could mistakenly categorize a susceptible person as immune; less sensitive commercial ELISAs are recommended for the purpose of screening if screening is indicated.

4. Hepatitis A

Serologic testing is required to confirm the diagnosis of hepatitis A. Acute hepatitis A virus (HAV) infection is confirmed during the acute or early convalescent phase of infection by the presence of **anti-HAV IgM** antibody in serum. IgM generally becomes detectable 5–10 days before the onset of symptoms and can persist for up to 6 months. The antibody test for total anti-HAV measures both anti-HAV IgG and anti-HAV IgM; therefore, a positive total anti-HAV test can be either an indicator of recent infection, past infection, or vaccination. If acute hepatitis A is suspected, anti-HAV IgM should be requested rather than total anti-HAV to avoid unnecessary re-testing.

5. Hepatitis B

Several markers in combination are used for accurate interpretation and staging of hepatitis B virus infection (see table below).

The presence of hepatitis B surface antigen, **HBsAg**, indicates that a person is infectious, regardless of whether the infection is acute or chronic. Screening for HBsAg to detect active (acute or chronic) hepatitis B virus (HBV) infection is recommended for all pregnant women at their first prenatal visit. The test may be repeated in the third trimester in women who are initially HbsAg negative and who are at increased risk of HBV infection during pregnancy. Routine screening for HBV infection in the general population is not recommended. Pre-vaccination screening in low-prevalence groups, such as health professionals in training, is usually not cost-effective.

Total anti-HBc (anti hepatitis B core antibody) develops in all HBV infections, but does not develop from vaccination. Anti-HBc indicates infection at some undefined time in the past. Anti-HBc generally persists for life and is not a serologic marker for acute infection.

IgM anti-HBc appears in persons with acute disease about the time of illness onset and indicates recent infection with HBV. IgM anti-HBc is generally detectable for four to six months after onset of illness and is the best serologic marker of acute HBV.

Anti-HBs (anti hepatitis B surface antibody) is a protective neutralizing antibody. The presence of anti-HBs following acute HBV infection generally indicates recovery and

immunity from reinfection. Anti-HBs develops in response to hepatitis B vaccine and can also be acquired through passive transfer by administration of HBIG. Post-vaccination testing, when recommended, should be performed 1–2 months following the third dose.

Interpreting the Hepatitis B Panel

Tests	Results	Interpretation
HBsAg anti-HBc anti-HBs	negative negative negative	Susceptible
HBsAg anti-HBc anti-HBs	negative negative positive with ≥ 10mIU/ml*	Immune due to vaccination
HBsAg anti-HBc anti-HBs	negative positive positive	Immune due to natural infection
HBsAg anti-HBc IgM anti-HBc anti-HBs	positive positive positive negative	Acutely infected
HBsAg anti-HBc IgM anti-HBc anti-HBs	positive positive negative negative	Chronically infected
HBsAg anti-HBc anti-HBs	negative positive negative	Four possible interpretations†

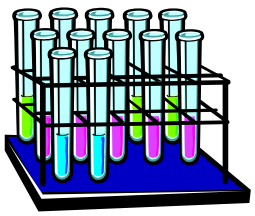
*Postvaccination testing, when it is recommended, should be performed 1–2 months after the last dose of vaccine. Infants born to HBsAg-positive mothers should be tested 3–9 months after the last dose.

†1.May be recovering from acute HBV infection
2. May be distantly immune and the test is not sensitive enough to detect a very low level of anti-HBS in serum
3. May be susceptible with a false positive anti-HBc
4. May be chronically infected and have an undetectable level of HBxAg present in the serum

HBeAg is a useful marker associated strongly with the number of infective HBV particles in the serum and a higher risk of infectivity. The IBL does not offer testing for HBeAg.

6. Hepatitis C

CDC has recommended that a person be considered to have serologic evidence of HCV infection only after an anti-HCV screening-test–positive result has been verified by a more specific serologic test (e.g., the recombinant immunoblot assay [RIBA®; Chiron Corporation, Emeryville, California]) or a nucleic acid test (NAT); however, laboratories may report a positive anti-HCV result based on a positive screening test result alone, and may not verify these results with more specific serologic or nucleic acid testing unless ordered by the requesting physician. Although the specificity of FDA-licensed or approved anti-HCV enzyme immunoassay screening test kits is >99%, among a population with a low prevalence of infection, such as among immunocompetent populations with anti-HCV prevalences <10% (e.g., volunteer blood donors, active duty and retired military personnel, persons in the general population, healthcare workers, or clients attending sexually transmitted disease clinics), the proportion of false-positive results may range from 15%–60%. Among immuno-compromised populations (e.g., hemodialysis patients), the proportion of false-positive results averages approximately 15%. For this reason, it is critical to not rely exclusively on anti-HCV screening-test–positive results to determine whether a person has been infected with HCV. Rather, screening-test–positive results should be verified with an independent supplemental test with high specificity.



References

ARUP Laboratories.* ARUP's Guide to Clinical Laboratory Testing: *Bordatella pertussis* Antibodies.http://www.aruplab.com/guides/clt/tests/clt_a120.jsp#1567190 .

Centers for Disease Control and Prevention.
1. Epidemiology and Prevention of Vaccine-Preventable Diseases. Eighth Edition. Jan. 2004. http://www.cdc.gov/nip/publications/pink/.
2. Guidelines for laboratory testing and result reporting of antibody to hepatitis C virus. http://www.cdc.gov/mmwr/PDF/rr/rr5203.pdf .
3. Manual for the Surveillance of Vaccine-Preventable Diseases 3rd Edition. 2002. http://www.cdc.gov/nip/publications

Turgeon, M. Immunology and Serology in Laboratory Medicine, Second Edition. Mosby: New York. 1996

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*Several commercial laboratories perform *Bordatella pertussis* antibody tests. The IDHW does not endorse any commercial laboratory.

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